Title:

Synthesis and biological evaluation of cyclopropyl analogues of fosmidomycin as potent Plasmodium falciparum growth inhibitors

Author(s): Vincent Devreux, J Wiesner, Jan Goeman, Johan Van der Eycken, H Jomaa and Serge Van Calenbergh

Source: JOURNAL OF MEDICINAL CHEMISTRY (2006), 49(8), 2656-2660, **DOI**: 10.1021/jm051177c

Synthesis and Biological Evaluation of Cyclopropyl Analogues of Fosmidomycin as Potent *P. falciparum* Growth Inhibitors

Vincent Devreux,[#,▽] *Jochen Wiesner,*[$] *Jan L. Goeman,*[▽] *Johan Van der Eycken,*[▽] *Hassan Jomaa*[$] *and Serge Van Calenbergh*[#,*]

[#] Laboratory for Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, B-9000 Gent, Belgium,

[▽] Laboratory of Organic and Bioorganic Chemistry, Department of Organic Chemistry, Faculty of Sciences, Ghent University, Krijgslaan 281 (S.4), B-9000 Gent, Belgium, and

[$] Universitätsklinkum Giessen und Marburg, Institut für Klinische Chemie und Pathobiochemie, Gaffkystrasse 11, 35392 Giessen, Germany.

* To whom correspondence should be addressed. Phone: +32 9 264 81 24. Fax + 32 9 264 81 46. E-MAIL: Serge.VanCalenbergh@UGent.be.

RECEIVED DATE (to be automatically inserted after your manuscript is accepted if required according to the journal that you are submitting your paper to)

ABSTRACT A series of fosmidomycin analogues featuring restricted conformational mobility has been synthesized and evaluated as inhibitors of 1-deoxy-D-xylulose-5-phosphate (DOXP) reductoisomerase and as growth inhibitors of *P. falciparum*. The enantiomerically pure *trans* cyclopropyl *N*-acetyl

analogue **3b** showed comparable inhibitory activity as fosmidomycin toward *E. coli* DOXP reductoisomerase and proved equally active when tested *in vitro* for *P. falciparum* growth inhibition. Conversely, the α-phenyl *cis*-cyclopropyl analogue **4** showed virtually no inhibition of the enzyme.

KEYWORDS: FOSMIDOMYCIN, CYCLOPROPYL PHOSPHONATE, CONFORMATIONAL RESTRICTION, 2*C*-METHYL-D-ERITHRYTOL 4-PHOSPHATE PHOSPHATE PATHWAY, 1-DEOXY-D-XYLULOSE-5-PHOSPHATE REDUCTOISOMERASE

MANUSCRIPT TEXT

Malaria is fatal for approximately 1.5 to 2.7 million people each year. Among other reasons, the increasing resistance to prevalent drugs such as chloroquine allows for a widespread emergence of *Plasmodium falciparum*, the causative agent of malaria tropica.

The discovery of the 2*C*-methyl-D-erithrytol 4-phosphate phosphate (MEP) pathway as a mevalonate-independent pathway for the biosynthesis of isoprenoids fueled research activities dealing with the characterization of the enzymes involved in this pathway and the search for inhibitors of these novel targets.[1,2] This MEP pathway is of particular interest since it is present in most bacteria, plants and the malaria parasite *P. falciparum*, but is absent in humans.

The natural antibiotics fosmidomycin (**1**) and FR-900098 (**2**) (Figure 1) posses high activities towards *P. falciparum in vitro.*[3] In addition, the antimalarial activity of fosmidomycin (**1**) was demonstrated in several recent clinical Phase II trials.[4-8]

The activity of these compounds is based on the inhibition of 1-deoxy-D-xylulose 4-phosphate reductoisomerase (DXR), the second enzyme in the non-mevalonate pathway. This enzyme mediates the conversion of 1-deoxy-D-xylulose 4-phosphate into 2*C*-methyl-D-erithrytol 4-phosphate.

Until now several X-ray crystallographic structures of DXR have been reported.[9,10] Additionally, Silber *et al.* recently published a study of the AFMoC force field as an enhanced *in silico* predictor of the binding affinity of ligands to DXR.[11]

Structural changes of fosmidomycin to enhance the activity of this lead towards *P. falciparum*, would provide critical information concerning structure-activity relationships of fosmidomycin. Much synthetic work has been dealing with phosphonate moiety alterations, such as ester prodrugs,[12-14] biphosphonates[15] or carboxylic acids.[16] Alternatively, several reports address hydroxamate moiety modifications, including benzoxazolone, benzothione, oxazopyridone or hydroxamic acid functionalities.[17,18] Interestingly, variations affecting the three carbon spacer are scarce. An important strategy to gather more knowledge of the structure-activity relationships of fosmidomycin involves the restriction of the rotational freedom of this carbon chain. This approach could be realized by incorporating the C-1 and C-2 atoms in a three-membered ring. Here we present the synthesis and biological activity of such novel cyclopropyl analogues (**3a-d**, **4**) of fosmidomycin. The synthetic strategy to generate the three-membered ring involves the intramolecular opening of an epoxide. This route was chosen above more obvious cyclopropanation schemes because it permits to employ readily available chiral epoxides for the synthesis of enantiomerically pure analogues. Moreover, even though the number of reports mentioning the catalytic enantioselective cyclopropanation of unsaturated phosphonates is growing, there is still no generally applicable highly enantioselective method to date. As it was envisioned that the use of a benzyl phosphonate would allow a clean final deprotection, dibenzylmethylphosphonate (**7**)[19] was chosen as starting material (Scheme 1). Regioselective opening of epoxide **8** with **7** under Lewis acid conditions afforded alcohol **9**. Special caution had to be taken to prevent side reactions, hence the temperature had to be monitored closely during the reaction. Deprotonation of alcohol **9** and subsequent elimination of the tosylate functionality yielded epoxide **5** as precursor for the formation of the three-membered ring. Ring closing was performed using a Lewis acid-assisted intramolecular epoxide ring opening, yielding alcohol **10**. Unfortunately, during this step an unavoidable partial benzyl deprotection occurred, which was also observed in step **a**. In accordance

with literature data,[20] the cyclopropane ring possessed the *trans*-configuration, which was proven via ^{1}H NMR coupling constants. Next, alcohol **10** was converted into tosylate **11** using a mild but very effective procedure published by Yoshida *et al.*,[21] which prevents further chlorine-assisted deprotection of the phosphonate. Subsequently, tosylate **11** was converted into Boc-benzyl protected hydroxylamine **12**, which, upon treatment with trifluoroacetic acid in dichloromethane, gave **13**. Acylation of the benzyloxyamine moiety using different reagents yielded protected hydroxylamides **14a-d**. Finally, hydrogenolysis of **14a-d** afforded the free phosphonates **3a-d**, which were purified via reversed phase HPLC.

Application of the above-mentioned strategy starting from the dibenzylbenzylphosphonate[22] led to the synthesis of the α-phenyl substituted cyclopropane analogue **4** (Scheme 2). As opposed to the unsubstituted cyclopropane ring only the *cis*-adduct was formed, based on both ^{1}H NMR coupling constants and a NOESY experiment. This result is in disagreement with a former study, which claims epoxide opening to yield exclusive formation of the *trans*-isomer irrespective of the α-substituent.[20]

The synthesized compounds were tested for inhibition of recombinant *E. coli* DXR because of the difficulties associated with the handling of the highly analogous *P. falciparum* enzyme. The conversion of DOXP to MEP by the enzyme was determined in an assay based on the NADPH dependency of the reaction.[1] The results are summarized in Table 1. The initially synthesized racemic *trans*-cyclopropane **3a** displayed activity in the submicromolar range, which prompted us to prepare the enantiomerically pure (1*R*,2*S*)-analogue **3b**. Compared to the racemic mixture this enantiomer displayed a remarkable activity enhancement, which indicates the preferred *trans*-cyclopropane stereochemistry to be (1*R*,2*S*). In fact, this eutomer represents the first synthesized analogue of fosmidomycin that exhibits similar activity on the DXR enzyme. Replacing the acetamide moiety of **3b** by a formamide moiety (**3c**) caused an 8-fold increase of the IC_{50} value, while homologation to the propionamide (**3d**) reduced the binding affinity by a factor of more than 150. The racemic α-phenyl-substituted *cis*-cyclopropane analogue **4** had no significant binding activity towards the DXR-enzyme. Based on the observed activity in the

enzyme assay, compounds **3b** and **3c** were evaluated for their inhibitory effect against intra-erythrocytic forms of *P. falciparum* (strains Dd2 and 3D7) using a semi-automated microdilution assay as described.[3] The growth of the parasites was monitored through the incorporation of tritium-labelled hypoxanthine. The results obtained are summarized in Table 2.

The (1*R*,2*S*)-*trans*-cyclopropane **3b** showed very promising *in vitro* antimalarial activity with both *P. falciparum* strains, performing equally as well as fosmidomycin. In accordance with the results obtained in the enzyme inhibition assay, the formamide analogue **3c** showed a 5-fold decreased *in vitro* antimalarial activity.

In summary a number of conformationally restricted fosmidomycin analogues have been synthesized. From this series, the (1*R*,2*S*)-*trans*-analogue with an *N*-hydroxy-*N*-acetamide group emerged as the best inhibitor of the *E. coli* DXR enzyme and proved equally as potent as fosmidomycin in inhibiting *P. falciparum* growth.

Experimental Section

General Methods and Materials.

Melting points were determined on a Reichert Heizbank type 184321 melting point apparatus calibrated with acetanilide (mp 114.5 °C) or an Electrothermal AI9100 digital melting point apparatus and are uncorrected. The ^{1}H, 13C and ^{31}P NMR spectra were recorded in $CDCl_3$, C_6D_6, MeOD or D_2O on a Bruker Avance 300 MHz-spectrometer. Chemical shifts are in parts per million with respect to TMS. RP-LC spectra were recorded on a Agilent 1100 series LC/MS spectrometer with quaternary pump and DAD detector. Mass spectroscopy spectra were recorded on a single quadrupole spectrometer type VL. Optical rotations were measured on a Perkin Elmer 241 polarimeter. Silicagel (60Å, 0.063-0.200 mm) was purchased from BioSolve. Preparative reversed phase chromatography was performed on a Phenomenex Luna C-15 (2) 5 μm particle (21.20 × 250 mm) column using 5 mM NH_4OAc solution and MeCN as solvents. Enantiomeric excess calculation was performed on a Diacel Chiralcel OD-H column

using hexane and absolute EtOH as isocratic solvents (flow rate = 1 mL/min). Tetrahydrofuran was distilled from sodium on benzophenone; dichloromethane, pyridine and Et_3N were distilled from CaH_2 unless otherwise stated. All other solvents and chemicals were used as purchased unless otherwise stated. *n*-BuLi was titrated from diphenylacetic acid prior to use. Reactions were performed under Ar-atmosphere in oven-dried flasks unless otherwise stated.

Dibenzyl 3-hydroxy-4-(*p*-toluenesulfonyloxy)butanephosphonate (9a).

A 2.3 M solution of *n*-BuLi in hexanes (20.6 mL, 47.4 mmol) was added dropwise to a stirred solution of dibenzyl methylphosphonate (13.1 g, 47.4 mmol) in dry THF (94 mL) while the temperature was kept below -70°C. After 15 min of stirring a solution of glycidyl tosylate (7.2 g, 34.5 mmol) was added dropwise, followed by $BF_3.OEt_2$ (8.0 mL, 63.2 mmol) under continuous monitoring of temperature below -70°C. The mixture was stirred for 7 h at -78 °C, after which the reaction was quenched with a NH_4Cl-solution (200 mL) and allowed to warm to room temperature. The aqueous phase was extracted three times with CH_2Cl_2 (200 mL). The combined organic fractions were dried on anhydrous $MgSO_4$, the solids were filtered and solvents were removed under reduced pressure. The residual oil was purified via column chromatography (pentane/CH_2Cl_2/acetone: 6/3/1) giving 8.7 g of a thick grey-white oil in a yield of 50%.

^{1}H NMR (300.13 MHz, $CDCl_3$) δ 1.57-1.97 (4H, m), 2.42 (3H, s), 3.77-3.92 (3H, m), 4.91 (1H, dd, J = 11.9 and 8.4 Hz), 4.92 (1H, dd, J = 11.9 and 8.3 Hz), 5.01 (2H, dd, 11.9 and 9.2), 7.29-7.33 (12H, m), 7.76 (2H, d, J = 8.3 Hz); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 21.6 (CH_3), 21.9 (P-CH_2, $^1J_{PC}$ = 142.2 Hz), 25.8 (-CH_2, $^2J_{PC}$ = 4.9 Hz), 67.4 (-OCH_2, $^2J_{PC}$ = 6.0 Hz), 68.8 (-OCH, $^3J_{PC}$ = 13.2 Hz), 72.9 (-OCH_2), 128.0 (=CH), 128.5 (=CH), 128.6 (=CH), 129.9 (=CH), 132.7 (=CH), 136.2 (=C, $^3J_{PC}$ = 5.5 Hz), 145.0 (=C); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ 33.6; ESMS *m/z* 505 ([M]+H^+).

Dibenzyl 3,4-epoxybutanephosphonate (5a).

To a stirred solution of **9a** (2.4 g, 4.76 mmol) in dry THF (35 mL) was added KO*t*-Bu (694 mg, 6.18 mmol) in one portion at 0 °C and the mixture was stirred for 3 h at 0 °C. Saturated aqueous NH_4Cl (80 mL) was added and the mixture was allowed to warm to rt. The aqueous layer was extracted three times with CH_2Cl_2 (80 mL) and the combined organic fractions were dried on anhydrous $MgSO_4$, filtered and the solvents were removed under reduced pressure. The resulting oil was purified via column chromatography (hexane/acetone: 3/2) yielding 1.17 g of a pale yellow oil (74%).

^{1}H NMR (300.13 MHz, $CDCl_3$) δ 1.75-1.93 (4H, m), 2.41 (1H, dd, J = 4.8 and 2.6 Hz), 2.68-2.70 (1H, m), 2.90 (1H, ddt, J = 6.1, 4.1 and 2.3 Hz), 4.96 (1H, dd, J = 11.8 and 8.6 Hz), 4.96 (1H, dd, 11.8 and 8.1 Hz), 5.05 (2H, dd, J = 11.8 and 9.0 Hz), 7.34 (10H, m); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 22.3 (P-CH_2, $^1J_{PC}$ = 142.7 Hz), 25.5 (-CH_2, $^2J_{PC}$ = 4.4 Hz), 47.0 (-OCH_2), 51.7 (-OCH, $^3J_{PC}$ = 19.8 Hz), 67.2 (-OCH_2, $^2J_{PC}$ = 6.6 Hz), 67.3 (-OCH_2, $^2J_{PC}$ = 6.5 Hz), 128.0 (=CH), 128.5 (=CH), 128.7 (=CH), 136.3 (=C, $^3J_{PC}$ = 6.0 Hz); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ 32.2; ESMS *m/z* 333 ([M]+H^+), 355([M]+Na^+), 687 ([2M]+Na^+).

Dibenzyl 2-(hydroxymethyl)-1-cyclopropylphosphonate (10a).

A 2.4 M solution of *n*-BuLi in hexanes (5.11 mL, 12.3 mmol) was added dropwise to a stirred solution of **5a** (3.4 g, 10.2 mmol) in dry THF at -78 °C. After 5 min of stirring $BF_3.OEt_2$ (2.58 mL, 20.5 mmol) was added dropwise, after which the mixture was stirred for 15 min at -78 °C. The reaction was quenched with saturated aqueous NH_4Cl (250 mL) and the aqueous phase was extracted twice with CH_2Cl_2 (250 mL) and twice with $CHCl_3$ (250 mL). The combined organic fractions were dried on anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residual oil was purified via column chromatography (hexane/CH_2Cl_2/acetone: 2/9/9), giving 1.56 g of a thick colorless oil (46%).

^{1}H NMR (C_6D_6, 300.13 MHz) δ 0.46 (1H, dddd, J = 11.6, 9.2, 5.7 and 4.4 Hz), 0.74 (1H, dddd, J = 9.2, 5.5, 5.5 and 5.2 Hz), 1.10 (1H, dddd, J = 17.7, 8.4, 5.9 and 4.4 Hz), 1.69 (dddddd, J = 16.0, 8.4, 6.4, 5.4, 5.4 and 5.4 Hz), 1.79 (1H, br s), 3.03 (1H, dd, J = 11.5 and 6.5 Hz), 3.35 (1H, ddd, J = 11.5, 5.4 and 2.0

Hz), 4.89 (1H, dd, J = 11.8 and 8.5 Hz), 4.95 (1H, dd, J = 11.8 and 8.5 Hz), 4.96 (1H, dd, J = 11.9 and 8.5 Hz), 5.04 (1H, dd, J = 12.2 and 8.5 Hz), 7.00-7.27 (10H, m); ^{13}C NMR (75.47 MHz, C_6D_6) δ 8.3 (-CH_2, $^2J_{PC}$ = 5.5 Hz), 9.4 (P-CH, $^1J_{PC}$ = 195.9 Hz), 19.7 (-CH, $^2J_{PC}$ = 4.4 Hz), 64.8 (-OCH_2, $^3J_{PC}$ = 3.8 Hz), 67.6 (-OCH_2, $^2J_{PC}$ = 6.1 Hz), 67.7 (-OCH_2, $^2J_{PC}$ = 6.5 Hz), 128.1 (=CH), 128.6 (=CH), 128.8 (=CH), 136.6 (=C); ^{31}P NMR (121.50 MHz, C_6D_6) δ 45.1; ESMS *m/z* 333 ([M]+H^+), 665 ([2M]+H^+), 687 ([2M]+Na^+).

Dibenzyl 2-(*p*-toluenesulfonyloxymethyl)-1-cyclopropylphosphonate (11a).

To a solution of alcohol **10a** (380 mg, 1.143 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C was added dry Et_3N (480 µL, 3.43 mmol) and Me_3N.HCl (33 mg, 0.343 mmol). A suspension of TsCl (327 mg, 1.715 mmol) in dry CH_2Cl_2 was added at 0°C, and the mixture was stirred for 1 h at the same temperature. Saturated aqueous NH_4Cl (70 mL) was added and the aqueous layer was extracted 3 times with CH_2Cl_2 (70 mL). The combined organic fractions were dried on anhydrous $MgSO_4$, filtered and the solvents were removed under reduced pressure. The residual oil was purified via column chromatography (hexane/CH_2Cl_2/acetone: 3/1/1) yielding 450 mg of a viscous colorless oil (81%).

^{1}H NMR (300.13 MHz, $CDCl_3$) 0.60-0.82 (2H, m), δ 0.96-1.08 (1H, m), 1.68 (1H, dddddd, J = 15.4, 8.4, 6.9, 6.8, 5.4 and 5.4 Hz), 2.34 (3H, s), 3.67 (1H, dd, J = 10.5 and 7.0 Hz), 3.81 (1H, ddd, J = 10.6, 6.7 and 1.6 Hz), 4.89 (2H, dd, J = 11.9 and 8.4 Hz), 4.95 (1H, dd, J = 11.4 and 8.4 Hz), 4.96 (1H, dd, J = 11.4 and 8.3 Hz), 7.22 (12H, m), 7.65 (2H, d, J = 8.3 Hz); ^{13}C NMR (75.47 MHz, $CDCl_3$) 8.7 (-CH_2, $^2J_{PC}$ = 4.9 Hz), δ 10.1 (P-CH, $^1J_{PC}$ = 195.9 Hz), 15.9 (-CH, $^2J_{PC}$ = 3.9 Hz), 21.6 (-CH_3), 67.5 (-OCH_2, $^2J_{PC}$ = 6.1 Hz), 67.7 (-OCH_2, $^2J_{PC}$ = 6.1 Hz), 72.1 (-OCH_2, $^3J_{PC}$ = 4.0 Hz), 127.9 (=CH), 128.5 (=CH), 128.6 (=CH), 129.9 (=CH), 133.0 (=C), 136.2 (=C, $^3J_{PC}$ = 6.1 Hz), 136.3 (=C, $^3J_{PC}$ = 6.0 Hz), 145.0 (S-C=); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ 29.0; ESMS *m/z* 487 ([M]+H^+).

Dibenzyl 2-[*N*-(benzyloxy),*N*-(*t*-butoxycarbonyl)aminomethyl]-cyclopropylphosphonate (12a).

NaH (47.3 mg, 0.987 mmol, 50% in mineral oil) was added to a solution of *tert*-butyl N-(benzyloxy)carbamate (220 mg, 0.987 mmol) in anhydrous DMF (5 mL) and the mixture was stirred for 30 min at rt. A solution of **11a** (400 mg, 0.822 mmol) in dry DMF (5 mL) was added dropwise to the solution containing the deprotonated carbamate and the reation mixture was stirred for 3 h at 50 °C. The reaction was quenched with saturated aqueous NH_4Cl (40 mL) and the water phase was extracted 3 times with CH_2Cl_2 (40 mL). The combined organic fractions were dried on anhydrous $MgSO_4$, filtered and the solvents were removed under reduced pressure. The residual oil was purified via column chromatography (hexane/CH_2Cl_2/acetone: 4/1/1) yielding 352 mg of compound **12a** as a colorless oil (80%).

^{1}H NMR (300.13 MHz, $CDCl_3$) δ 0.72-0.89 (2H, m), 1.10 (1H, dddd, J = 18.2, 8.5, 5.6 and 4.2 Hz), 1.46 (9H, s), 1.73 (1H, dddddd, J = 15.7, 8.3, 6.9, 6.9, 5.5 and 5.5 Hz), 3.29 (2H, d, J = 6.7 Hz), 4.83 (2H, s), 4.97 (2H, dd, J = 11.8 and 7.9 Hz), 5.03 (1H, dd, J = 11.9 and 8.3 Hz), 5.03 (1H, dd, J = 12.0 and 7.9 Hz), 7.30-7.38 (15H, m); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 9.3 (-CH_2, $^2J_{PC}$ = 4.9 Hz), 10.0 (P-CH, $^1J_{PC}$ = 195.4 Hz), 15.6 (-CH, $^2J_{PC}$ = 3.8 Hz), 28.3 (-CH_3), 52.8 (-NCH_2, $^3J_{PC}$ = 4.8 Hz), 67.3 (-OCH_2, $^2J_{PC}$ = 6.0 Hz), 67.4 (-OCH_2, $^2J_{PC}$ = 5.5 Hz), 77.2 (-OCH_2), 81.6 (O-C), 127.8 (=CH), 127.9 (=CH), 128.3 (=CH), 128.5 (=CH), 128.6 (=CH), 129.4 (=CH), 135.6 (=C), 136.5 (=C, $^3J_{PC}$ = 6.6 Hz), 136.6 (=C, $^3J_{PC}$ = 6.6 Hz), 156.6 (N-C=O); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ 30.7; ESMS *m/z* 438 ([M]-Boc+2H$^+$), 538 ([M]+H$^+$).

Dibenzyl 2-[*N*-(benzyloxy)aminomethyl]-cyclopropylphosphonate (13a).

TFA (2.58 mL, 33.6 mmol) was added dropwise to a solution of **12a** (350 mg, 0.651 mmol) in dry CH_2Cl_2 (6 mL) at 0 °C and the mixture was stirred for 45 min at 0 °C. The mixture was transferred into a separation funnel and saturated aqueous $NaHCO_3$ (40 mL) was added. The aqueous layer was extracted twice with CH_2Cl_2 (40 mL) and twice with $CHCl_3$ (40 mL). The combined organic layers were dried on $MgSO_4$, filtered and the solvents were removed under reduced pressure yielding 256 mg of pale yellow solid in a yield of 90%.

mp 40-41 °C; ^{1}H NMR (300.13 MHz, $CDCl_3$) δ0.61-0.74 (2H, m), 1.04-1.15 (1H, m), 1.60 (1H, dddddd, J = 16.1, 8.4, 6.7, 6.7, 5.4 and 5.4 Hz); 2.66 (1H, dd, J = 13.2 and 6.8 Hz), 2.75 (1H, ddd, J = 13.2, 6.7 and 1.7 Hz), 4.62 (2H, s), 4.94 (2H, dd, J = 11.8 and 8.1 Hz), 5.00 (1H, dd, J = 11.9 and 8.3 Hz), 5.02 (1H, dd, J = 11.9 and 8.3 Hz), 7.21-7.31 (15H, m); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ9.3 (-CH_2, $^{2}J_{PC}$ = 5.5 Hz), 10.0 (P-CH, $^{1}J_{PC}$ = 196.0 Hz), 15.9 (-CH, $^{2}J_{PC}$ = 3.8 Hz), 55.3 (-NCH_2, $^{3}J_{PC}$ = 3.8 Hz), 67.3 (-OCH_2, $^{2}J_{PC}$ = 6.0 Hz), 67.4 (-OCH_2, $^{2}J_{PC}$ = 6.1 Hz), 76.3 (-OCH_2), 127.8 (=CH), 128.3 (=CH), 128.4 (=CH), 128.5 (=CH), 136.6 (=C, $^{3}J_{PC}$ = 6.1 Hz), 136.6 (=C, $^{3}J_{PC}$ = 6.1 Hz), 137.8 (=C); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ31.1; ESMS *m/z* 438 ([M]+H^+), 460 ([M]+Na^+), 875 ([2M]+H^+).

Dibenzyl 2-[*N*-acetyl,*N*-(benzyloxy)aminomethyl]-cyclopropylphosphonate (14a).

Amine **13a** (256 mg, 0.586 mmol) was dissolved in dry pyridine (6 mL). Ac_2O (615 µL, 6.51 mmol) was added dropwise and the mixture was stirred overnight at rt. The mixture was transferred to a separation funnel and a 1 M HCl-solution (70 mL) was added. The aqueous layer was extracted twice with CH_2Cl_2 (70 mL) and twice with $CHCl_3$ (70 mL). The combined organic fractions were dried on anhydrous $MgSO_4$, filtered and the solvents were removed on rotary evaporation. The residual oil was dissolved in CH_2Cl_2 (40 mL), saturated aqueous $NaHCO_3$ (40 mL) was added, the layers were separated and the aqueous phase was extracted twice with CH_2Cl_2 (40 mL) and once with $CHCl_3$ (40 mL). The combined organic layers were dried on anhydrous $MgSO_4$, filtered and the solvents were removed under reduced pressure. The residual oil was purified via column chromatography (hexane/CH_2Cl_2/MeOH: 50/48/2) giving 253 mg of a pale yellow oil in an overall yield of 90%.

^{1}H NMR (300.13 MHz, $CDCl_3$) δ0.81 (1H, dddd, J = 12.0, 9.4, 5.3, 4.3 Hz), 0.89-0.97 (1H, m), 1.11 (1H, dddd, J = 18.2, 8.4, 5.7 and 4.4 Hz), 1.73 (1H, dddddd, J = 15.7, 8.4, 7.0, 6.8, 5.4 and 5.4 Hz), 2.00 (3H, s), 3.47 (1H, dd, J = 14.9 and 7.4 Hz), 3.53 (1H, ddd, J = 14.9, 6.6 and 1.8 Hz), 4.79 (2H, s), 4.95 (1H, dd, J = 12.1 and 7.7 Hz); 4.97 (1H, dd, J = 11.8 and 8.4 Hz), 5.02 (1H, dd, J = 11.8 and 8.4 Hz), 5.03 (1H, dd, J = 12.3 and 8.4 Hz), 7.19-7.30 (15H, m); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ9.4 (-CH_2, $^{2}J_{PC}$ = 5.0 Hz), 10.4 (P-CH, $^{1}J_{PC}$ = 185.4 Hz), 15.8 (-CH, $^{2}J_{PC}$ = 3.8 Hz), 20.4 (-CH_3), 50.0 (-NCH_2),

67.3 (-OCH_2, $^2J_{PC}$ = 6.0 Hz) 67.4 (-OCH_2, $^2J_{PC}$ = 6.0 Hz), 76.9 (-OCH_2), 127.7 (=CH), 127.8 (=CH), 128.3 (=CH), 128.5 (=CH), 128.7 (=CH), 129.0 (=CH), 129.1 (=CH), 134.4 (=C), 136.5 (=C, $^3J_{PC}$ = 6.1 Hz), 136.6 (=C, $^3J_{PC}$ = 6.6 Hz), 167.8 (N-C=O); ^{31}P NMR (121.50 MHz, $CDCl_3$) δ 30.5; ESMS *m/z* 480 ([M]+H^+).

2-[*N*-(acetyl),*N*-(hydroxy)aminomethyl]-cyclopropylphosphonic acid (3a).

To a solution of **14a** (253 mg, 0.528 mmol) in MeOH (5 mL) was added Pd/C (100 mg, 10%). The reaction was placed under H_2 (1 atm) and was stirred overnight at rt. The mixture was filtered over celite and the celite was washed with portions of MeOH. The filtrate was evaporated under reduced pressure; the residual oil was dissolved in a minimal amount of water and lyophilised. The resulting solid was purified via reversed phase HPLC using a gradient elution of 5 mM NH_4OAc solution to MeCN in 20 min. The appropriate fractions were lyophilised, giving 80 mg of an amber amorphous solid in a yield of 72%.

mp 100-102 °C; ^{1}H NMR (300.13 MHz, MeOD) δ 0.45 (1H, m), 0.57 (1H, m), 0.77 (1H, m), 1.27 (1H, m), 1.95 (3H, s), 3.14-3.21 (1H, m), 3.53 (1H, dd, *J* = 14.3 and 5.3 Hz); ^{13}C NMR (75.47 MHz, MeOD) δ 9.6 (-CH_2, $^2J_{PC}$ = 5.1 Hz), 14.6 (-CH, $^1J_{PC}$ = 177.8 Hz), 15.8 (-CH, $^2J_{PC}$ = 6.1 Hz), 20.2 (-CH_3), 52.7 (-NCH_2, $^3J_{PC}$ = 3.3 Hz), 173.6 (N-C=O); ^{31}P NMR (121.50 MHz, D_2O) δ 22.7; ESMS *m/z* 210 ([M]+H^+).

ACKNOWLEDGMENT This study was supported by grants from the European Commission (QLK2-CT-2002-00887) and INTAS (03-51-4077). Technical assistance was provided by Dajana Henschker.

SUPPORTING INFORMATION Experimental details (^{1}H, ^{13}C, ^{31}P NMR, MS, HPLC) for intermediates (**9b**, **5b**, **10b-13b**, **14b-d**, **6**, **16-19** and **21**) and final products (**3b-d**, **4**) and determination of the relative configuration of the cyclopropane ring. This material is available free of charge via Internet at http://pubs.acs.org.

REFERENCES

(1) Kuzuyama, T.; Shimizu, T.; Takahashi, S.; Seto, H. Fosmidomycin, a specific inhibitor of 1-deoxy-D-xylulose 5-phosphate reductoisomerase in the nonmevalonate pathway for terpenoid biosynthesis. *Tetrahedron Lett.* **1998,** *39*, 7913-7916.

(2) Zeidler, J.; Schwender, J.; Muller, C.; Wiesner, J.; Weidemeyer, C.; Beck, E.; Jomaa, H.; Lichtenthaler, H. K. Inhibition of the non-mevalonate 1-deoxy-D-xylulose-5-phosphate pathway of plant isoprenoid biosynthesis by fosmidomycin. *Z. Naturforsch., C: Biosci.* **1998,** *53*, 980-986.

(3) Jomaa, H.; Wiesner, J.; Sanderbrand, S.; Altincicek, B.; Weidemeyer, C.; Hintz, M.; Turbachova, I.; Eberl, M.; Zeidler, J.; Lichtenthaler, H. K.; Soldati, D.; Beck, E. Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs. *Science* **1999,** *285*, 1573-1576.

(4) Lell, B.; Ruangweerayut, R.; Wiesner, J.; Missinou, M. A.; Schindler, A.; Baranek, T.; Hintz, M.; Hutchinson, D.; Jomaa, H.; Kremsner, P. G. Fosmidomycin, a novel chemotherapeutic agent for malaria. *Antimicrob. Agents Chemother.* **2003,** *47*, 735-738.

(5) Missinou, M., Borrmann, S., Schindler, A., Issifou, S., Adegnika, A. A., Matsiegui, P. B., Binder, R., Lell, B., Wiesner, J., Baranek, T., Jomaa, H., Kremsner, P. G. Fosmidomycin for malaria. *Lancet.* **2002,** 360, 1941-1942.

(6) Borrmann, S.; Issifou, S.; Esser, G.; Adegnika, A. A.; Ramharter, M.; Matsiegui, P. B.; Oyakhirome, S.; Mawili-Mboumba, D. P.; Missinou, M. A.; Kun, J. F. J.; Jomaa, H.; Kremsner, P. G. Fosmidomycin-clindamycin for the treatment of Plasmodium falciparum malaria. *J. Infect. Dis.* **2004,** *190*, 1534-1540.

(7) Borrmann, S.; Adegnika, A. A.; Matsiegui, P. B.; Issifou, S.; Schindler, A.; Mawili-Mboumba, D. P.; Baranek, T.; Wiesner, J.; Jomaa, H.; Kremsner, P. G. Fosmidomycin-clindamycin for plasmodium falciparum infections in African children. *J. Infect. Dis.* **2004,** *189*, 901-908.

(8) Borrmann, S., Adegnika, A. A., Moussavou, F., Oyakhirome, S., Esser, G., Matsiegui, P. B., Ramharter, M., Lundgren, I., Kombila, M., Issifou, S., Hutchinson, D., Wiesner, J., Jomaa, H., Kremsner, P. G. Short-course regimens of artesunate-fosmidomycin in treatment of uncomplicated Plasmodium falciparum malaria. *Antimicrob Agents Chemother.* **2005,** *49*, 3749-3754

(9) Steinbacher, S.; Kaiser, J.; Eisenreich, W.; Huber, R.; Bacher, A.; Rohdich, F. Structural basis of fosmidomycin action revealed by the complex with 2-C-methyl-D-erythritol 4-phosphate synthase (IspC) - Implications for the catalytic mechanism and anti-malaria drug development. *J. Biol. Chem.* **2003,** *278*, 18401-18407.

(10) Mac Sweeney, A.; Lange, R.; Fernandes, R. P. M.; Schulz, H.; Dale, G. E.; Douangamath, A.; Proteau, P. J.; Oefner, C. The crystal structure of E. coli 1-deoxy-D-xylulose-5-phosphate reductoisomerase in a ternary complex with the antimalarial compound fosmidomycin and NADPH reveals a tight-binding closed enzyme conformation. *J. Mol. Biol.* **2005,** *345*, 115-127.

(11) Silber, K.; Heidler, P.; Kurz, T.; Klebe, G. AFMoC enhances predictivity of 3D QSAR: A case study with DOXP-reductoisomerase. *J. Med. Chem.* **2005,** *48*, 3547-3563.

(12) Ortmann, R.; Wiesner, J.; Reichenberg, A.; Henschker, D.; Beck, E.; Jomaa, H.; Schlitzer, M. Alkoxycarbonyloxyethyl ester prodrugs of FR900098 with improved in vivo antimalarial activity. *Arch. Pharm.* **2005,** *338*, 305-314.

(13) Ortmann, R.; Wiesner, J.; Reichenberg, A.; Henschker, D.; Beck, E.; Jomaa, H.; Schlitzer, M. Acyloxyalkyl ester Prodrugs of FR900098 with improved in vivo anti-malarial activity. *Bioorg. Med. Chem. Lett.* **2003,** *13*, 2163-2166.

(14) Reichenberg, A.; Wiesner, J.; Weidemeyer, C.; Dreiseidler, E.; Sanderbrand, S.; Altincicek, B.; Beck, E.; Schlitzer, M.; Jomaa, H. Diaryl ester prodrugs of FR900098 with improved in vivo antimalarial activity. *Bioorg. Med. Chem. Lett.* **2001,** *11*, 833-835.

(15) Ling, Y.; Sahota, G.; Odeh, S.; Chan, J. M. W.; Araujo, F. G.; Moreno, S. N. J.; Oldfield, E. Bisphosphonate inhibitors of Toxoplasma gondi growth: In vitro, QSAR, and in vivo investigations. *J. Med. Chem.* **2005,** *48*, 3130-3140.

(16) Kurz, T.; Geffken, D.; Wackendorff, C. Carboxylic acid analogues of fosmidomycin. *Z. Naturforsch., B: Chem. Sci.* **2003,** *58*, 457-461.

(17) Courtois, M.; Mincheva, Z.; Andreu, F.; Rideau, M.; Viaud-Massuard, M. C. Synthesis and biological evaluation with plant cells of new fosmidomycin analogues containing a benzoxazolone or oxazolopyridinone ring. *J. Enzyme Inhib. Med. Chem.* **2004,** *19*, 559-565.

(18) Kuntz, L.; Tritsch, D.; Grosdemange-Billiard, C.; Hemmerlin, A.; Willem, A.; Bacht, T. J.; Rohmer, M. Isoprenoid biosynthesis as a target for antibacterial and antiparasitic drugs: phosphonohydroxamic acids as inhibitors of deoxyxylulose phosphate reducto-isomerase. *Biochem. J.* **2005,** *386*, 127-135.

(19) Kissling, R. M.; Gagne, M. R. Alkali metal alkoxide clusters: Convenient catalysts for the synthesis of methylphosphonates. *J. Org. Chem.* **1999,** *64*, 1585-1590.

(20) Hah, J. H.; Gil, J. M.; Oh, D. Y. The stereoselective synthesis of cyclopropylphosphonate analogs of nucleotides. *Tetrahedron Lett.* **1999,** *40*, 8235-8238.

(21) Yoshida, Y.; Sakakura, Y.; Aso, N.; Okada, S.; Tanabe, Y. Practical and efficient methods for sulfonylation of alcohols using Ts(Ms)Cl/Et3N and catalytic Me3N center dot HCl as combined base: Promising alternative to traditional pyridine. *Tetrahedron* **1999,** *55*, 2183-2192.

(22) Saady, M.; Lebeau, L.; Mioskowski, C. First Use of Benzyl Phosphites in the Michaelis-Arbuzov Reaction - Synthesis of Monophosphate, Diphosphate, and Triphosphate Analogs. *Helv. Chim. Acta* **1995,** *78*, 670-678.

TABLES

Table 1. Inhibition of recombinant *E. coli* DXR

Compound	**IC_{50} (μM)**
Fosmidomycin	0.048
FR900098	0.058
3a	0.160
3b	0.050
3c	0.313
3d	> 3.0
4	> 30

Table 2. *In vitro* growth inhibition of the *P. falciparum* strains Dd2 and 3D7

Compound	**IC_{50} (μM)**	
	Dd2	**3D7**
Fosmidomycin	0.48	0.40
FR900098	0.28	0.24
3b	0.48	0.32
3c	2.0	2.1

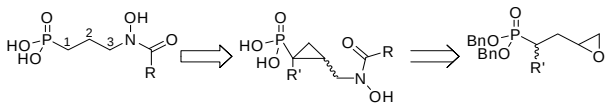
FIGURE LEGENDS

Figure 1. Targeted fosmidomycin analogues and synthetic strategy

Scheme 1. Synthesis of the unsubstituted *trans*-cyclopropyl fosmidomycin analogues

Scheme 2. Synthesis of substituted *cis*-cyclopropyl fosmidomycin analogue

Figure 1

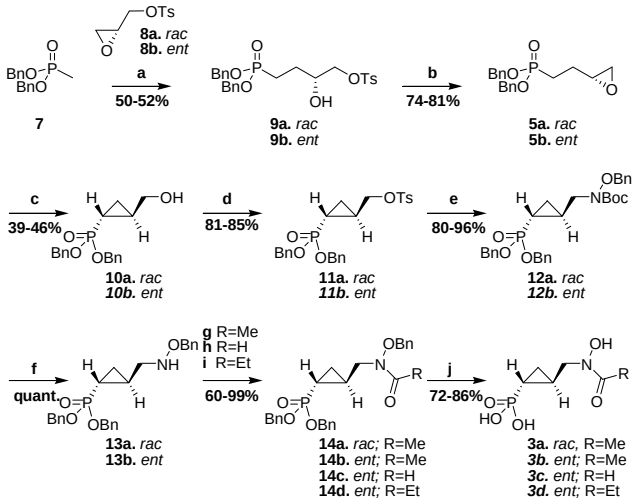


1. R = H
2. R = CH_3

3a. *rac trans*; R = Me; R' = H
3b. (1*R*, 2*S*); R = Me; R' = H
3c. (1*R*, 2*S*)R = R' = H
3d. (1*R*, 2*S*) R = Et, R' = H
4. *rac cis*; R = Me; R' = Phenyl

5. R' = H
6. R' = Phenyl

Scheme 1



7

8a. *rac*
8b. *ent*

a 50-52%

9a. *rac*
9b. *ent*

b 74-81%

5a. *rac*
5b. *ent*

c 39-46%

10a. *rac*
10b. *ent*

d 81-85%

11a. *rac*
11b. *ent*

e 80-96%

12a. *rac*
12b. *ent*

f quant.

13a. *rac*
13b. *ent*

g R=Me
h R=H
i R=Et
60-99%

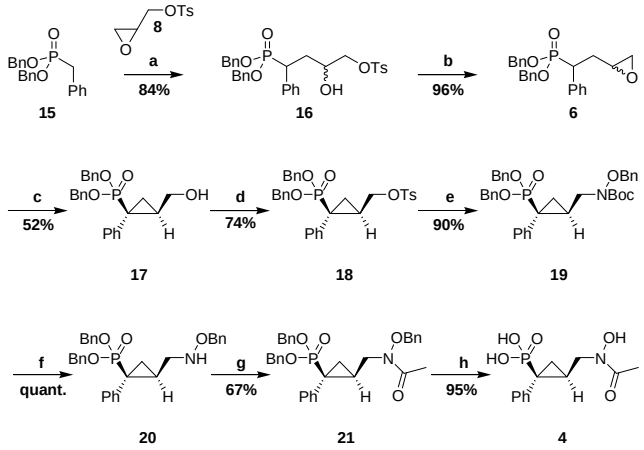
14a. *rac;* R=Me
14b. *ent;* R=Me
14c. *ent;* R=H
14d. *ent;* R=Et

j 72-86%

3a. *rac,* R=Me
3b. *ent;* R=Me
3c. *ent;* R=H
3d. *ent;* R=Et

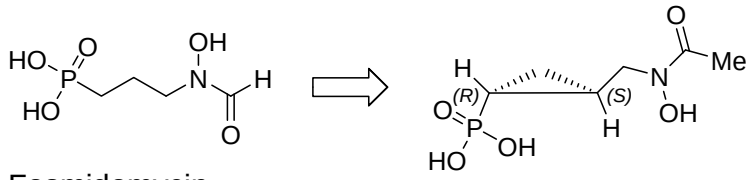
[a]Reagents and conditions: (**a**) (i) *n*-BuLi, THF, < -70 °C, (ii) **8**, < -70 °C, (iii) $BF_3.OEt_2$; (**b**) KO*t*Bu, THF, 0 °C; (**c**) (i) *n*-BuLi, THF, -78 °C, (ii) $BF_3.OEt_2$, -78 °C; (**d**) TsCl, $Me_3N.HCl$, Et_3N, 0 °C; (**e**) BocNHOBn, NaH, DMF, 50 °C; (**f**) TFA, CH_2Cl_2, 0 °C; (**g**) Ac_2O, pyridine, rt; (**h**) HCOOH, DCC, pyridine, $CHCl_3$, 0 °C to rt; (**i**) $(C_2H_5CO)_2O$; pyridine, rt; (**j**) H_2, Pd/C, rt

Scheme 2



[a] Reagents and conditions: (**a**) (i) *n*-BuLi, THF, < -70 °C, (ii) **8**, < -70 °C, (iii) $BF_3.OEt_2$ < -70 °C; (**b**) KO*t*Bu, THF, 0 °C; (**c**) (i) *n*-BuLi, THF, -78 °C, (ii) $BF_3.OEt_2$, -78 °C; (**d**) TsCl, $Me_3N.HCl$, Et_3N, 0 °C; (**e**) BocNHOBn, NaH, DMF, 50 °C; (**f**) TFA, CH_2Cl_2, 0 °C; (**g**) Ac_2O, pyridine, rt; (**h**) H_2, Pd/C, rt

TOC Graphic



Fosmidomycin

IC_{50} for Dd2 strain = 0.48 nM IC_{50} = 0.48 nM